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The effects of extrusion conditions on the nutritional value of soybean meal for rainbow trout (*Oncorhynchus mykiss*)☆

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Abstract

Feed ingredients and complete feeds are exposed to varying degrees of heating during manufacturing and processing. Heat treatment of soybeans is necessary to de-nature endogenous trypsin inhibitors and maximize nutritional value, but overheating can damage protein and reduce nutritional value. This study was conducted to determine the effects of extrusion cooking, the primary method of aquafeed production, on the nutritional value of fish meal and soybean meal based diets. A $2\times2\times2$ factorial treatment design was used with pre-cooking (+ or −), time in extruder barrel (18 or 37 s), and extruder temperature (93 or 127 °C) as the fixed factors. These conditions were selected to achieve both under and overcooking of the soy protein, given the limitations of the equipment. A twin-screw cooking extruder (Buhler, DNDL-44) was used to produce the eight experimental diets and each was fed to triplicate groups of 40 g trout for 84 days. Trypsin inhibitor levels (TIU), protein dispersibility index (PDI), nitrogen solubility index (NSI), and apparent digestibility coefficients (ADC) of protein, organic matter, lipid, energy and carbohydrate were measured for each diet. Commercially purchased, solvent extracted SBM contained 5100 TIU/g, and after pre-cooking through the extruder with barrel temperatures of 127 °C for 17 s, contained 2300 TIU/g. The diets contained less than the detectable limit of 2000 TIU/g, probably due to a combination of dilution and processing effects. PDI values of the diets suggest that pre-cooking SE-SBM decreased protein value, however, neither PDI nor NSI values were correlated to weight gain. There was no significant effect of pre-cooking or extruder temperature on feed intake or weight gain, but time in the extruder barrel significantly affected feed intake and weight gain; longer extrusion time significantly decreased feed intake and weight gain. Higher temperature in the extruder barrel significantly improved FCR. Pre-cooking SE-SBM before inclusion in the diet significantly improved the ADC for organic matter, energy, and carbohydrates. These results demonstrate the importance of extruder processing conditions on fish performance, and indicate high temperature (127 °C) and short time in the extruder barrel results in the greatest weight gain of rainbow trout.

Keywords: Diet processing; Extrusion; Soybean processing; Rainbow trout

1. Introduction

Numerous studies have been conducted on replacing all or a portion of fish meal in aquaculture diets with soybean meal, and the results with carnivorous fish species, e.g., Atlantic salmon (*Salmo salar*), rainbow

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trout (*Onchorynchus mykiss*), sea bass (*Dicentrarchus labrax*), yellowtail (*Seriola quinqueradiata*), all indicate that the upper range of replacement before fish performance or health is reduced is about 25% of the fish meal, or between 10% and 15% of the diet (Fowler, 1980; Reinitz, 1980; Arnesen et al., 1990; Krogdahl et al., 1994; Arndt et al., 1999; Refstie et al., 2000, 2001; Vielma et al., 2000). Fish performance is affected below the level of substitution at which specific amino acids in soybean meal are limiting to the fish (NRC, 1993). Anti-nutritional factors in soybean meal, including trypsin inhibitors (Krogdahl et al., 1994; Arndt et al., 1999), insoluble carbohydrates (Arnesen et al., 1990) saponins (Bureau et al., 1998), and proteins that cause an immune response (Rumsey et al., 1995) have been sighted as causes for this common effect. There is little dispute that in Atlantic salmon and rainbow trout, soybean meal-containing diets cause changes of intestinal morphology (van den Ingh et al., 1991; Rumsey et al., 1995; Refstie et al., 2000). Despite this effect and the known effects of trypsin inhibitors on protein digestibility, there is little evidence to suggest that soy protein is poorly digested by salmonids; in fact apparent digestibility values for soybean meal protein are relatively high (Sugiura et al., 1998; Refstie et al., 2000; Sugiura and Hardy, 2000).

Extrusion technology has been used in the feed industry for almost one century (Hardy and Barrows, 2000). Extrusion cooking is accomplished by using a combination of moisture, pressure, temperature and mechanical shear (Barrows and Hardy, 2001). Mild extrusion cooking conditions can enhance digestibility of plant protein (Srihara and Alexander, 1984, Hakansson et al., 1987). As a result, extrusion normally improves nutrient digestibility, palatability, pellet durability, water stability and pellet storage life (Barrows and Hardy, 2000). In turn, the growth performance and feed efficiency of animals fed extruded feeds is thought to improve. Extrusion increased the digestibility of dry matter in soybean meal from 75% to 78%, and digestibility of energy from 79% to 82%, as measured *in vivo* using rainbow trout (Cheng and Hardy, 2003). Protein digestibility was not improved, but was already 98% in this study. Bjork et al. (1984) reported that extruding wheat flour increased soluble fiber from 40% to 75%. Increasing soluble fiber content is thought to improve fiber digestibility, and thus increase digestible energy.

Many chemical tests are used to gauge the effects of heat processing on the nutritional value of soybean meal (Vohra and Kratzer, 1991; Hsu and Satter, 1995). Some tests are used to determine if soybean meals are under-heated while others detect overheating. Under-heated meals presumably contain higher levels of trypsin inhibitors than properly heated meals. Soy products containing less than 5 mg/g (mg bovine trypsin inhibited/g feed) are thought to have been sufficiently heated for rainbow trout (Olli and Krogdahl, 1994), common carp (*Cyprinus carpio*) (Viola et al., 1983), channel catfish (*Ictalurus punctatus*) (Wilson and Poe, 1985) and Atlantic salmon (Olli et al., 1994). Overheated meals are undesirable because overheating may damage protein and decrease protein digestibility and amino acid availability. Specifically, lysine is bound with sugars during the Maillard reaction resulting in the deoxyketosyl compound of lysine which is unavailable to trout and other terrestrial animals (Plakas et al., 1988). Lysine availability decreased in white sturgeon (*Acipenser transmontanus*) in feeds heated excessively (Deng et al., 2005).

Protein solubility index (PSI) is a test used to detect overheating of soybean meal. As soybean meal is heated, protein solubility and PSI decrease. Arndt et al. (1999) found that trypsin inhibitor levels decreased rapidly as duration of autoclaving increased, and that protein solubility followed a similar but delayed pattern. PSI values were 98% in uncooked defatted soy flour, decreased to 70% after 10 and 20 min of autoclaving, and decreased to 33% after 40 min. After 120 min, solubility was 18%. Trypsin units inhibited, in contrast, were 10% of initial values after 10 min of autoclaving, and 1% after 20 min. Thus the degree of heating required to lower trypsin inhibitor levels is less than the degree required to lower protein solubility.

Recently, nutritionists have become aware that while PSI is an appropriate test to identify overcooked soybean meal, it is not useful to distinguish good from excellent meals, i.e., soybean meals cooked to the point of optimum nutritional value. Another test, protein dispersability index (PDI), has been proposed as a test that can distinguish nutritional quality in soybean meals having PSI values over 85%. This is critical because over 90% of soybean meals in the USA have PSI values over 80–85%, yet they are not equivalent in nutritional value to fish. PDI has been demonstrated to be a simple and effective procedure in assessment of the quality of heat-treated soybeans (Hsu and Satter, 1995). The Nitrogen Solubility Index is a slight modification of the PDI procedure (AOCS, 1983), and is used frequently in the food processing industry (Zhang and Liu, 2005; Kahn et al., 2003).

Conventional soybean meal is subjected to heat treatment during production to reduce trypsin inhibitor levels. With the domination of high temperature extrusion technology in the aquafeed industry, the potential for overheating soybean meal and thereby reducing nutritional value to fish exists. The first objective of this study was to determine if the effect of heating during ingredient and diet processing were additive, and if it affected fish performance. The second objective was to evaluate various chemical tests on diets with high soybean meal content for their predictive value of performance of rainbow trout. The third objective was to determine if extrusion processing conditions (time and temperature) could be optimized to enhance growth rate and feed efficiency of rainbow trout.

2. Materials and methods

2.1. Experimental design

Diets contained 52% solvent extracted soybean meal (SE-SBM) to increase the likelihood that the effects of extrusion conditions on SE-SBM or fish performance could be detected (Table 1). Diets were formulated to meet or exceed all known nutrient requirements for rainbow trout (NRC, 1993). A 2 by 2 by 2 factorial treatment design was used with pre-cooking of the SE-SBM (yes or no), extruder barrel temperature (93 or 127 °C), exposure time of the complete diet in the extruder barrel (18 or 37 s) as the main effects resulting in eight experimental diets. Four of the experimental diets contained SE-SBM that was pre-cooked and the other four diets contained the same lot of SE-SBM that had not been exposed to heat after initial processing at the commercial plant. Pre-cooking the SE-SBM was used as a way to add additional heating to the SE-SBM without heating other ingredients. The time and temperatures chosen provided as much and as little heat or time exposure as possible, while still maintaining a quality pellet. This was thought to provide a practical level of both under and overcooking of the soy protein.

Table 1
Ingredient composition (g/kg) of experimental diet

Ingredient	
Fish meal [a]	242
Solvent-extracted soybean meal [b]	525
Wheat flour	73
Fish oil, incorporated [c]	91
Fish oil, after pelleting [c]	60
Vitamin premix [d]	6
Trace mineral premix [e]	1
Stay-C	2

[a] Menhaden Special Select, Omega Proteins Corporation, Houston, TX.

[b] Either pre-cooked or uncooked as required by treatment.

[c] Menhaden, Omega Proteins Corporation, Houston, TX.

[d] Contributed per kilogram of diet: vitamin A (as retinol palmitate), 10,000 IU; vitamin D_3, 720 IU; vitamin E (as DL-∝-tocopheryl-acetate), 530 IU; niacin, 330 mg; calcium pantothenate, 160 mg; riboflavin, 80 mg; thiamin mononitrate, 50 mg; pyridoxine hydrochloride, 45 mg; menadione sodium bisulfate, 25 mg; folacin, 13 mg; biotin, 1 mg; vitamin B_{12}, 30 ug.

[e] Tblfn4Contributed in mg/kg of diet: zinc, 37; manganese, 10; iodine, 5; copper, 1.

2.2. Fish and culture

Rainbow trout (mean weight 40.5 g) were selected, counted in groups of 25 fish, weighed, and placed into twenty four, 150-L fiberglass tanks (two replicate tanks per dietary treatment), each supplied with 6 L/min of untreated, constant temperature (14.5 °C), spring water at the Hagerman Fish Culture Experiment Station, University of Idaho. A domesticated strain of rainbow trout (House Creek strain, College of Southern Idaho) was used. A fixed photoperiod, controlled by timers and fluorescent lights, was followed (14 hours daylight:10 hours dark). Fish were fed three times per day, six days per week to apparent satiation for a period of 70 days. Apparent satiation was considered achieved when the fish would no longer aggressively consume feed. There were no wasted pellets for any of the diets during the study, so feed intake values represent actual feed consumed. The experimental protocol was approved by the University of Idaho's Animal Care and Use Committee.

To determine the effect of extrusion processing conditions on the digestibility of dietary nutrients the same eight experimental diets were tested following the growth trial. Fish from each treatment were pooled and redistributed into two 500 l tanks, with 20 fish per tank, for a total of sixteen tanks. The larger tanks used in the digestibility phase of the project were needed to accommodate the larger fish size, and this required a reduction from three replicates per diet in the growth phase to two replicates per diet in the digestibility phase. The fish were fed their respective diets to apparent satiation twice daily for five days. All diets contained 0.1% yttrium oxide as an inert marker. Feces were collected by hand stripping from all fish within each tank. Feces were pooled by tank and stored at −20 °C until analyzed. Apparent digestibility coefficients (ADC) were determined for organic matter, lipid, energy, carbohydrate, and protein.

2.3. Diet Preparation

SE-SBM was pre-cooked using a twin-screw cooking extruder (DNDL-44, Buhler AG, Uzwil, Switzerland) with an 18 s exposure to 127 °C in the extruder barrel. The 3.0 mm SE-SBM pellets were then dried in a pulse bed drier (Buhler AG, Uzwil, Switzerland) for 25 min at 102 °C with a 10 min cooling period, resulting in final moisture levels less than 10%. The SE-SBM was ground to a particle size of <200 μm using an air-swept pulverizer (Model 18H, Jacobsen, Minneapolis, MN). All of the experimental diets were processed using the same extruder and drier used to pre-cook the SE-SBM. The time that the diet mixture was exposed to heat in the extruder barrel was controlled by changing the feed rate of the diet mash into the steam conditioner and thus the extruder. A rate of 600 rpm on the feeder (Model K2VT35, K-Tron North America, Pitman, NJ) resulted in 37 s of heat exposure and a setting of 1200 rpm on the feeder resulted in 18 s of heat exposure in the extruder. Pressure at the die head varied across treatments due to changing extruder temperature and retention times. All diets were preconditioned with steam (Extru-tech, Sabetha KS) prior to entering the extruder. The extruder has 6 barrel sections and temperature was controlled by heating section 2, 3, 4, and 5 for diets processed at 127 °C. Only section 2 was heated for diets processed at 93 °C. Barrel sections 1 and 6 were cooled under both processing conditions. After the diets had dried they were top-coated with oil (6%) at ambient pressure using a horizontal paddle mixer (Model 210, Rapids Machinery Co., Marion, IA). Ten kg of each diet was ground, 0.1% yttrium was added, and re-pelleted by cold extrusion (EXDC(F)S-60 extruder, LCI, Inc., Charlotte, NC) for determinations of ADC's.

2.4. Chemical analyses

Feed and fecal samples were dried, and analyzed using AOAC (1995) methods for proximate composition, with the exception of protein and crude lipid. Dried samples were finely ground by mortar and pestle and analyzed for crude protein (total nitrogen × 6.25) using a LECO FP-428 nitrogen analyzer (LECO Instruments, St. Joseph, MI). Crude fat was analyzed using a soxhlet extraction apparatus (Soxtec System HT, Foss Tecator AB, Hoganas, Sweden) with methylene chloride as the extracting solvent, and ash by incineration at 550 °C in a muffle furnace. Energy content of the samples was determined using a Parr bomb calorimeter (Parr Instrument Co., Moline, IL). Yttrium analyses were conducted at the University of Idaho Analytical Sciences Laboratory, Moscow, ID, using an Optima 3200 radial inductively-coupled plasma atomic emission spectrometer (Perkin-Elmer Corp., Norwalk, CT).

Trypsin inhibitor units (TIU), protein dispersibility index (PDI) and nitrogen solubility index (NSI) were determined for the solvent extracted soybean products and each feed using AOCS (1983) Official methods Ba 12–75, Ba 10–65 and Ba 11–65, respectively. NSI is a similar method to PDI, but utilizes a slower mixing speed.

Table 2
Mean of Trypsin Inhibitor Activity, Protein Dispersibility Index (PDI) of diets processed with varied conditions

	Time s	Temperature °C	PDI %	NSI %	Extrusion Pressure[a], PSI
Pre-cook: no[b]	18	93	14.8	15.1	175
	18	127	13.1	13.0	170
	37	93	12.5	13.9	90
	37	127	13.4	12.4	116
Pre-cook: yes[b]	18	93	14.5	13.1	187
	18	127	14.7	12.4	170
	37	93	15.7	13.7	145
	37	127	16.6	13.3	102
Pre-cook					
No[c]			13.55	13.60	138
Yes[c]			15.37	13.12	151
Time					
18 s[c]			14.27	13.40	175
37 s[c]			14.55	13.32	113
Temperature					
93 °C[c]			14.37	13.95	149
127 °C[c]			14.45	12.77	139

[a] Measured at die head.
[b] $n=2$.
[c] $n=8$.

2.5. Calculation of performance indices and apparent digestibility coefficients

Fish performance indices were calculated using the following formulae:

$$\text{Specific growth rate (SGR)} = (\ln \text{final weight} - \ln \text{initial wt}) / \text{duration of experiment (days)}$$

$$\text{Apparent feed conversion ratio (FCR)} = \text{feed intake (dryweight)} / \text{body weight gain (wetweight)}.$$

Apparent digestibility coefficients (ADC) of diets for organic matter, lipid carbohydrate and protein were calculated using Yttrium oxide as the inert marker and the following formula:

$$\text{Diet ADC}(\%) = 100 \times [1 - (\%\ \text{Yttrium in diet}/\%\ \text{Yttrium in feces}) \times (\%\ \text{nutrient in feces}/\%\ \text{nutrient in diets})]$$

(Cho and Slinger, 1979).

2.6. Statistical analyses

Fish performance and nutrient digestibility data were analyzed using the general linear models procedure of

Table 3
Mean of growth performance of trout fed diets processed with varied conditions

	s	°C	Weight gain, (g/fish)	SGR (g/fish/day)	FCR	Feed Intake (g/fish)
Single factor ANOVA						
No [a]	18	93	197.3	2.81	1.06	209.6
	18	127	204.3	2.91	1.02	208.2
	37	93	193.0	2.75	1.08	207.1
	37	127	181.0	2.59	1.05	190.0
Pooled SE			13.99	0.21	0.06	19.93
Yes [a]	18	93	189.3	2.71	1.09	206.9
	18	127	198.7	2.83	1.07	213.1
	37	93	190.0	2.71	1.07	203.5
	37	127	193.3	2.76	1.05	195.7
Pooled SE			14.76	0.82	0.09	11.21
Diet, *P* of >*F* value			0.01	0.01	0.01	0.01
R^2			0.90	0.90	0.94	0.78
C.V.			6.80	6.79	3.72	6.10

[a] $n=3$.

the Statistical Analysis System (SAS, 1988). Data was analyzed as a 2 by 2 by 2 factorial treatment design, and interactive effects were determined. Tank mean values were considered units of observation for statistical tests, and mean values were considered significantly different when $P<0.05$.

3. Results

3.1. Pre-cooking of SE-SBM ingredient

Pre-cooking of SE-SBM decreased TIU, PDI and NSI suggesting an improvement of soybean meal quality. Pre-cooking for 18 s at 127 °C at a pressure of 260 PSI decreased TIU, PDI and NSI values from 5100, 28.9 and 25.6 to 2300 TIU/mg, 21.6% and 11.2%, respectively.

3.2. TIU, PDI and NSI of diets after processing

After the diets were mixed and processed, the TIU levels of all diets were below the detection limit of the assay (<2000 TIU/mg). Both PDI and NSI levels were reduced in the complete feeds compared to SE-SBM. The lowest PDI values were observed for the no pre-cooking treatments, and the lowest NSI values were observed for the high extruder temperature (127 °C) treatments (Table 2).

Table 4
Three factor analysis growth performance of trout fed diets processed with varied conditions

	Weight Gain, (g/fish)	SGR (g/fish/day)	FCR	Feed Intake (g/fish)
Three factor ANOVA				
Pre-cook				
No [a]	192.8	2.75	1.06	204.8
Yes [a]	193.9	2.76	1.05	203.7
Pooled SE	3.98	0.05	0.01	4.16
Time				
18 s [a]	197.4	2.82	1.06	209.4
37 s [a]	189.3	2.71	1.05	199.1
Pooled SE	3.57	0.05	0.02	3.55
Temperature				
93 °C [a]	192.4	2.75	1.08	206.8
127 °C [a]	194.3	2.78	1.04	201.7
Pooled SE	1.20	0.05	0.01	4.05
P of >F value				
Pre-cook	0.75	0.76	0.41	0.75
Time	0.03	0.03	0.48	0.01
Temperature	0.57	0.55	0.01	0.14
Pre-cook * Time [b]	0.10	0.11	0.04	0.99
Time * Temperature [b]	0.08	0.10	0.64	0.03
R^2	.46	0.44	.45	0.54
C.V.	4.2	4.2	3.2	3.9

[a] $n=12$.
[b] Other interactions were not significant.

Table 5
Three factor analysis of apparent digestibility coefficients for organic matter, lipid, energy, and carbohydrate of diets processed with varied conditions

	Organic Matter	Lipid	Energy	CHO	Protein
Three factor ANOVA					
Pre-cook					
No [a]	74.0	98.7	74.6	30.2	91.3
Yes [a]	75.8	97.9	76.6	36.7	92.3
Pooled SE	0.84	0.20	1.14	2.10	0.31
Time					
18 s [a]	74.5	98.3	75.8	32.8	91.0
37 s [a]	75.3	98.3	75.4	34.2	91.5
Pooled SE	1.06	0.14	1.27	2.56	0.33
Temperature					
93 °C [a]	74.6	98.0	76.3	33.8	91.0
127 °C [a]	75.2	98.6	74.9	33.2	91.6
Pooled SE	1.00	0.19	1.20	0.69	0.18
Probability of >F value					
Pre-cook	0.01	0.21	0.02	0.01	0.99
Time	0.17	0.86	0.66	0.32	0.31
Temperature	0.29	0.17	0.12	0.65	0.19
Pre-cook * Time [b]	0.06	0.07	0.54	0.94	0.06
Pre-cook * Temperature [b]	0.01	0.39	0.01	0.01	0.08
Model	0.01	0.15	0.01	0.01	0.02
R^2	.89	.51	.80	.86	.71
C.V.	1.5	0.9	2.1	8.1	1.1

[a] $n=12$.
[b] Other interactions were not significant.

3.3. Growth performance

Despite the high level of SE-SBM in the diet, trout gained of over 400% of their intital wieght in all treatments during the 12-week study indicating good growth (Table 3). Pre-cooking SE-SBM before extrusion did not affect weight gain, SGR, feed intake or FCR (Table 4). There was an effect of time in the extruder barrel on weight gain, with 18 s resulting in an average weight gain of 197 g/f compared to 189 g/f for the fish fed diets processed for 37 s in the extruder barrel. There was a trend for a significant interaction of time and temperature ($P=0.08$). This interaction is apparent because when extruder time was short (18 s), increased temperature resulted in an increase in weight gain from 193.3 g/f to 201.5 g/f. When extruder time was long (37 s), however, weight gain decreased from 191.5 g/f to 187.1 as processing temperature increased (Table 3).

Feed intake was affected by the amount of time the diet spent in the extruder (Table 4). Higher feed intakes were observed for the fish fed the diets exposed to the extruder for only 18 s compared to fish fed diets held in the extruder for 37 s, 209.4 g fish^{-1} and 199.1 g fish^{-1}, respectively. Pre-cooking or extruder temperature did not affect feed intake.

FCR was significantly improved for fish fed diets processed at the higher temperature (Table 4). Fish fed the diets extruded at 127 °C had FCR of 1.04 compared to 1.08 for the fish fed the diets extruded at 93 °C. There was an interaction effect of pre-cooking and time (Table 4). When SE-SBM was not pre-cooked before processing, the shorter extrusion time resulted in better FCR. When the SE-SBM was pre-cooked, the longer extrusion time resulted in better FCR (Table 3).

Table 6
Single factor analysis of apparent digestibility coefficients for organic matter, lipid, energy, and carbohydrate of diets processed with varied conditions

Pre-cook	Time s	Temperature °C	Organic matter	Lipid	Energy	CHO	Protein
Single factor ANOVA							
No[a]	18	93	75.7	98.8	77.4	33.5	92.1
	18	127	72.7	99.4	71.6	25.5	91.0
	37	93	76.2	98.3	77.2	35.9	91.7
	37	127	71.5	98.3	72.1	26.1	90.2
Pooled SE			1.63	0.53	1.87	1.65	0.68
Yes[a]	18	93	71.7	96.0	76.0	34.3	88.0
	18	127	77.9	98.8	78.0	37.8	92.7
	37	93	74.9	98.7	74.4	31.5	91.7
	37	127	78.8	98.0	78.0	43.3	92.5
Pooled SE			0.60	0.68	0.58	3.02	1.01
P of >F value							
Diet			0.01	0.01	0.01	0.01	0.01
R^2			0.97	0.94	0.98	0.98	0.98
C.V.			2.12	2.48	1.65	3.27	1.58

[a] $n=3$.

3.4. Apparent digestibility of the diets

The ADC for organic matter, energy, carbohydrate were all improved by pre-cooking the SE-SBM before extrusion (Table 5). Pre-cooking significantly increased the ADC for organic matter, energy, and carbohydrate from, 74.0 to 75.8%, 74.6 to 76.6%, and 30.2 to 37.6%, respectively. There was no effect of processing condition on lipid ADC. There was a significant interaction ($P<0.01$) of pre-cooking and extruder temperature on ADC for organic matter, energy, and carbohydrate (Table 5). When the SE-SBM was not pre-cooked the ADC for carbohydrate for fish diets processed at the 93 °C was 34.7%, compared to 25.8% for fish fed diets processed at 127 °C. When the SE-SBM was pre-cooked, the ADC for carbohydrate increased from 32.9% for fish diets processed at the 93 °C to 40.5% for fish fed diets processed at 127 °C (Table 6).

4. Discussion

Weight gain of fish in the current study was reduced compared to fish fed diets with lower levels of SE-SBM, but were still over 400% in the 12 week study. In different studies, trout reared at the same facility and fed diets containing just 15% SE-SBM gained 450% of initial weight in 9 weeks (Gaylord et al., 2006), and trout fed diets with only 9% SE-SBM gained over 600% of initial weight in 12 weeks (Stone et al., 2005). Lower growth rates were expected in the present study when feeding diets containing 52.5% SE-SBM, but these high levels were considered necessary to accentuate the effects of processing on fish performance.

Extrusion conditions significantly affected weight gain, feed intake and FCR of rainbow trout in this study. Similar results were reported for channel catfish (Peres et al., 2003) and carp (Viola et al., 1983). A beneficial effect of heating of soybean meal on weight gain, feed intake and feed efficiency of channel catfish was observed (Peres et al., 2003). Growth rates of carp were reduced when fed diets containing under-heated soybean meal (Viola et al., 1983). The magnitudes of differences among treatments for catfish and carp were greater than observed for trout, but un-heated soybeans were used with catfish and carp and commercial, SE-SBM was used in the present study. In both the catfish and carp studies cold-processed diets containing varied soybean meal products were fed. This is in contrast to the present study where the additive effect of heating was investigated.

Feed intake was lower for fish fed diets that had been in the extruder barrel 37 s compared to 18 s. A similar effect of time was also observed on weight gain, with larger gains observed for fish fed feed processed for 18 s. The increase in weight gain may be a direct effect of extruder time on feed intake. Processing time did not affect ADC. Viola et al. (1983) concluded that slightly overheated soybean meal, may have inadequate lysine levels due to availability associated with the Maillard reaction.

Sorensen et al. (2002) reported that extrusion temperature had no effect on ADC for protein or energy for Atlantic salmon. Those findings agree with the results of the present study in which extruder temperature alone did not affect ADC for organic matter, lipid, energy, or carbohydrate. Higher levels of plant ingredients were used in present study compared to the fish meal and wheat diets, 50% and 30% respectively, used by Sorensen et al. (2002). Extrusion cooking alone, regardless of conditions, was not sufficient to render all the energy available to the trout, but there was an effect in the present study of pre-cooking on the ADC for organic matter, energy and carbohydrate. Pre-cooking did increase these ADC's, however, but the effect was not reflected by a significant effect of pre-cooking on growth performance. This could be due to the study only lasting 12 weeks, and had diets containing pre-cooked SE-SBM been fed for entire an production cycle, an effect may have been observed. However, over 400% weight gain was recorded in the present study and if the effect was present, it ought to have been detected.

An interaction of extruder temperature by pre-cooking on ADC's was observed; values for organic matter, energy and carbohydrate increased as extruder temperature increased when the SE-SBM was pre-cooked. When the SE-SBM was not pre-cooked, an increase in extruder temperature resulted in a decrease in ADC. While other studies have documented an increase in ADC of plant-based ingredients or diets due to extrusion cooking (Cheng and Hardy, 2003; Allan and Booth, 2004), the additive effect of pre-cooking an ingredient had not been investigated. An additive effect of precooking and extruder temperature on the ADC for carbohydrate was observed in the present study. Fish fed diets processed at 93 °C including non or pre-cooked SE-SBM was 34.7% and 32.9%, respectively, indicating no effect of precooking. When the diets were processed at 127 °C, the carbohydrate ADC for diets containing pre-cooked SE-SBM increased to 40.5%, compared to only 25.8% for diets with SE-SBM that was not pre-cooked. When the effects of pre-cooking and extruder temperature were combined, the digestibility of carbohydrate increased which in turn influenced the ADC's for organic matter and energy. The observed additive effect of pre-cooking and extruder temperature may be due to the high dietary inclusion level of SE-SBM in this study, and may not be detected in short term studies with lower levels of SE-SBM in the diet.

Pre-cooking improved chemical scores relating to nutritional quality of SE-SBM by decreasing TIU, PDI and NSI values. van der Ven et al. (2005) demonstrated that a combination of pressure and temperature was effective in reducing trypsin inhibitor activity, but increased pressure alone was not effective. Dilution of the SE-SBM during diet mixing reduced TIU levels in diets to approximately 2652 TIU/mg, and extrusion further reduced TIU levels below detection limits for all diets (<2000 TIU/mg). Processing of all feeds in the current study included both temperature and pressure increases, and this decreased TIU to below detectable levels. Pressures from 90 to 260 PSI were observed in the present study, with diets processed at shorter retention time receiving the highest pressures (Table 2). Although pre-cooking improved chemical scores of soybean quality, extrusion processing without pre-cooking resulted in similar improvement in diet quality. Improvements in fish performance due to optimized extrusion conditions were apparently not due to residual trypsin inhibitor levels.

PDI values of the complete diets in the current study ranged from 12.5 to 16.6%. Batal et al. (2000) reported that values less than 45% indicate adequate heat treatment of soybeans for chicks. This is somewhat higher, however, than the recommendation of 15 to 30% of the National Soybean Processors Association (Batal et al., 2000). Working with Holstein heifers, Hsu and Satter (1995) found a PDI of even lower than the recommended level to be optimal, 9–11%. Zhu et al. (1996) reported that extrusion temperature decreased PDI values of whole soybeans from 46% to 22% as temperature increased from 96 to 121 °C. In the present study, a similar temperature increase only changed PDI from 13.9 to 12.8%. This suggests that the extrusion conditions (pressure and moisture), and the degree of heat treatment prior to extrusion that were used resulted in maximum improvement in PDI. PDI has also been used as an indicator of quality when different processing methods for the production of soybean products are evaluated and values similar to those observed in the present study were reported (Wang et al., 2004). PDI values reported in this study, however, were not as low as those reported to be optimal by Hsu and Satter (1995). This may be due to differences in the source of soybeans used in the two studies. Qin et al. (1998) found that the PDI values of Argentinean soybeans of responded to time of steam exposure and temperature differently than soybeans of Chinese origin. These authors concluded that soybeans of different origin required different processing conditions to optimize protein properties. NSI values showed similar trends to PDI values, except for the effect of extruder temperature. Similar to the response of FCR to extruder temperature, NSI values were improved when the diet was exposed to the higher temperature.

The first objective of this study was to evaluate the additive effect of heat processing on SE-SBM throughout ingredient production and diet processing. Even with additional heating provided by the pre-cooking treatment, weight gain during the 12 week study was not positively or negatively affected, but ADC's for carbohydrate, organic mater and energy were affected by pre-cooking. The second objective was to determine if various chemical tests could be used to predict performance of rainbow trout fed diets with high levels of SE-SBM. Since there were no apparent effect of extruder conditions on PDI or NSI, but there was an effect of extruder conditions on weight gain, FCR and feed intake, we can conclude that under the conditions of this study PDI and NSI are not suitable as indicators of the effect of processing conditions on fish performance. The third objective was to determine if extrusion processing conditions would affect performance of rainbow trout. Processing a diet with high temperature (127 °C) and short time in the extruder (18 s) resulted in the highest weight gains and lowest feed conversion ratios.

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